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CHROMOSOME STUDIES IV: A DEFICIENT SUPERNUMERARY ACCESSORY CHROMOSOME IN A MALE OF TETTIGIDEA PARVIPENNIS,
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Chromosome Studies IV: A Deficient Supernumerary Accessory Chromosome in a Male of *Tettigidea parvipennis*.

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THE normal number of chromosomes of the species of *Tettigidæ* ("grouse locusts") so far examined is thirteen and fourteen in the male and female, *i. e.*, twelve autosomes and either one or two X-chromosomes, respectively. (Robertson '16; Rayburn, '17.) The subject of this investigation is a specmen which possesses the secondary sexual characters of a male, yet has fourteen chromosomes. What makes the case still more striking is the fact that the extra chromosome is probably an X-chromosome. On measurement, however, it was found to be shorter than its mate by between one-fifth and one-fourth the normal length (figs. 15, 17, etc.). As will be seen, it is probably to be considered a supernumerary, but deficient, accessory chromosome.

Its occurrence in the individual is uniform for all cells found dividing, somatic or germinal. In somatic cells it is not so readily identified in every case yet the fact that the number fourteen may always be clearly counted indicates its presence when other means fail. The somatic cells found containing it were from "fat body," follicle wall, and *vasa efferentia* tissues. The chromosomes in these cells are large and widely separated. The drawings indicate their actual positions in most cases (figs. 1 to 5). No difficulty or doubt was had in making the count here. Of course, counts in somatic cells could be obtained only from tissues surrounding the testes,

since these were the only tissues that had been taken with the testes from the body of the animal at the time of cytological preservation. The carcass itself unfortunately was preserved in a weak solution of formalin. The fact however, that "fat body" cells and the cells of the testicular follicles and of the *vasa efferentia* show fourteen chromosomes is reason for thinking that this number would likely have been found in all somatic cells.

In the germ cells (spermatogonia, figs. 6 and 7; first spermatocytes, figs 8 to 21; and second spermatocytes, figs 22 to 27) this chromosome is uniformly present. It is here that, on account of the singular behavior of accessory chromosomes normally exhibited in spermatogenesis stages, a clew is first obtained as to the probable nature of this supernumerary body which resembles in every way an accessory chromosome. Its characteristics are as follows:

(1) It may be recognized along with the accessory in synapsis and growth stages by its condensed condition (figs. 8 to 16).

(2) In "bouquet" of synapsis, intermediate finely threaded or netlike, and early prophase stages of the first spermatocyte, it always lies near the wall of the nucleus, which is characteristic of the accessory chromosomes (figs. 8 to 13).

(3) It condenses ahead of the other chromosomes in prophases of the first spermatocyte (figs. 14 to 16).

(4) It has a tendency to pair side to side with the accessory during parasynapsis stages when other chromosomes are similarly pairing (figs. 8 to 11). This occurs in about from 65 per cent to 75 per cent of cells. At this time both the accessory and the supernumerary have the form of a right-handed corkscrew like spiral (fig. 8). At later stages, they lie not so closely together, though there is evidence, in many of the cells, that they are in some way attached to each other (figs. 14 to 19). The synapsis seems to be most intimate at the pointed ends, which, judging from the general position in the cell and from the direction of orientation of the ordinary chromosomes, I have a surmise may be the distal ends. Further evidence of the association or pairing which occurs in synapsis is afforded by their behavior in the first spermatocyte division (figs. 17, 18, 19). In some cases (fig. 17) they are traveling to the same pole closely approximated to each other; in other

cases (figs. 18, 19) they are traveling to opposite poles, but separating from each other much like the members of autosome pairs that have been in synapsis.

(5) When the conjugated pairs of chromosomes (tetrads) arrange themselves in the metaphase of the first spermatocyte division, the supernumerary body passes like an accessory, undivided to one pole, going either to the same pole as the accessory chromosome (figs. 17, 21), or to the opposite pole (figs. 18, 19, 20).

(6) The supernumerary appears in one-half of the second spermatocytes either with the accessory or without it (figs. 22 to 27).

(7) It divides in this division as does the accessory chromosome (figs. 26, 27). One-half the spermatids get it and one-half do not. Four sorts of spermatozoa accordingly are formed.

From the characteristics here given, it is evident that this body is probably an accessory chromosome. If it occurred in these cells in the absence of the normal accessory (No. 5*x*) it certainly would be taken for that body. It fills all the specifications for the accessory of *Tettigidea* except size. An idea of the difference in size may be gotten by comparing the lengths of the two chromosomes in figures 8, and 15 to 21. The proximal end, from which the spindle fiber springs, seems to be normal, but the distal end appears somewhat abnormal (figs. 14*a*, 15, 17, 18). It seems likely that this chromosome is a descendant of an X-chromosome which at some previous polar body formation failed to separate properly from its mate, losing thereby a portion from its distal end. An incomplete daughter chromosome such as this might result. In this connection it may be said that it behaves very much like the deficient No. 4 chromosome described in Studies III (Robertson, 1915), whose peculiar structure and behavior was accounted for in this way.

In the literature on supernumeraries, the nearest approach to this chromosome are the cases described by Stevens in *Diabrotica soror* and *D. 12-punctata* (1908 *b*, 1912 *a*) and in *Ceuthophilus* (1912 *b*). Normally in these species there is a large unpaired heterochromosome (accessory) in the male and two such in the female similar to what occurs in Orthoptera. Stevens found about 50 per cent of the males of the

Diabroticas to have from one to five supernumeraries and 20 per cent of the males in *Ceuthophilus* to have one or more supernumeraries. Her conclusion was that these bodies were probably related to X-chromosomes, descended from fragments of X-chromosomes that had arisen through irregularities in the maturation division at some previous time. Wilson regarded his supernumeraries in *Metapodius* as duplicates of the Y-chromosome, *i. e.*, of the smaller member of the idiochromosome pair. No such Y-chromosome has been described for Acrididæ or Locustidæ. In addition, the irregular behavior of this supernumerary body (*s*) with reference to the accessory chromosome (5*x*), in going either with it or not to one pole in the first spermatocyte, is evidence against its being a Y-chromosome. This was also true of the *Diabroticas* and *Ceuthophilus*. It differs, however, from the latter two cases in this: that no cell has been found showing this body dividing in first instead of the second spermatocyte division, as was true in a few cases in the *Diabroticas* and *Ceuthophilus*. Otherwise it behaves very much like the supernumeraries of these forms. It seems warrantable to consider it as belonging to the X-chromosome rather than the Y-chromosome category.

If this body is in reality an accessory chromosome, and one that has lost from the distal end one-fifth or one-fourth of its length, and if it is also to be considered functional in so far as the portion present is concerned, we may regard it as throwing some light upon the question as to how much and what portion of the accessory in the family *Tettigidæ* is concerned with sex-determination. In the first place, that the sex-determining genes are probably limited to only a portion of the accessory chromosome in the genus *Tettigidea*, at least is to be inferred from a comparison of the relative lengths of the chromosome in the different genera of the family to which the genus belongs, the chromosome varying from eight in *Acridium* to fourteen or fifteen in *Paratettix* and eighteen or nineteen in *Tettigidea*. This supposition is of course based upon the assumption, first of all, that the X-chromosome is to a large degree similar in constitution throughout the family. That this is true we have reason to believe from the fact that these genera which appear to be so closely related taxonomically possess chromosomes (both autosomes and allosomes) which have a very great degree of similarity in number, form, and relative size (Robert-

son, '16; Rayburn '17). Such could only arise if the chromosomes of the group are considered as genetically continuous and related. The same applies to the X-chromosome. Further evidence that sex chromosomes may be similary constituted throughout a series of related species is afforded by the work of Metz ('14, 16*a*, 16*b*, 16*c*, 16*d*) on the *Drosophilas*, which has shown that not only one plan of structure, in so far as relative sizes, etc., is concerned, may run through the chromosomes of each species of a genus, but, what is still more striking, that each gene has probably the same locus with reference to other genes along the length of the chromosome throughout the various species. If such be the case in the *Drosophilas* it seems a probability that the same might be true of the accessory chromosome among the genera and species of the *Tettigidæ*. Then, we are justified in surmising that, since in the genus *Acridium* the sex-determining chromosome has a value of 8, in the genus *Tettigidea*, where its length is relatively 19, only a portion of this chromosome, considerably less than half, *i. e.*, about eight nineteenths, could be concerned with sex-determination.

If a comparison of the sex chromosome in the genera of this family leads us to believe that the sex-determining portion in the genus *Tettigidea* is at most not greater than eight nineteenths of the total, in the case of the supernumerary accessory described in this "Study" we have evidence, it seems, which limits the size of this portion to a still greater degree, namely, between one fifth and one fourth the length of the chromosome. The reasons for concluding this are as follows: The presence of the chromosome in this particular animal has not produced a female; hence it must lack the part concerned with sex determination. Bridges ('16), in his splendid work upon nondisjunction of pairing sex chromosomes in *Drosophila amphilophila*, has shown that sex determination may be excluded from one portion of the X-chromosome at least, and therefore limited to a part of it. He found that a small portion of the X-chromosome in the region which carries the genes for "barring of the eye" and "forked condition of the bristles" became nonexistent genetically (pp. 150, 151). Females could exist with one such deficient X-chromosome. The sons of such heterozygous females were only one-half as numerous as the daughters, and did not possess the deficiency. The evidence warranted the conclusion that males could not exist with such

a deficient chromosome, Bridges' discovery has demonstrated, in the first place, that deficiencies may occur in X-chromosomes; in the second place, that the sex differentiators are limited to a part of the X-chromosome, for they did not occur in that portion as least of the chromosome which has to do with the above genes and which lies between the loci of the genes for "rudimentary wing" and "fused." Now if such be the case in *Drosophila,* then in *Tettigidea* we are justified in concluding that the sex determinants may likewise be limited to a portion of the chromosome, and since the animal which has here been described as possessing one normal and one short X is a male instead of a female, it seems safe to conclude that possibly the missing portion of this chromosome contains the determiners for sex, and further that in the genus *Tettigidea* at least these determiners lie near one end, probably the distal end, of the chromosome.

TIME OF APPEARANCE OF THE LONGITUDINAL SPLIT IN SOMATIC CHROMOSOMES

This discussion can not be closed without reference to the evidence afforded in figures 2 to 5 upon the question of the time at which division of the chromosomes in somatic mitoses takes place. In figures 2 and 3, late prophases of cells from the follicular wall, any portion of a chromosome which happens to run at all vertically shows distinctly in cross-section a secondary split at right angles to the very wide and apparent primary split. In figure 3, at the arrow point, such a portion of chromosome No. 4 is drawn. Most of the chromosomes here show the same. These cells have not yet entered upon the metaphase stage of division. Division, when it comes, will take place in the plane of the very apparent primary split. The secondary split here present can only be for the next again metaphase. This is shown much more conclusively in anaphases (figs. 4 and 5) from the same tissues. Here it is certain that division along the primary plane has taken place. The daughter chromosomes in traveling to the poles of the spindle show clearly the secondary split (fig. 5). It must be remembered that these chromosomes are entering upon the telophase stage of division, and go from that to the "resting" condition of the cell. Such a split is likely the forerunner of

the split which is most apparent in the prophase figures 2 and 3.

This is not the first time that such a phenomenon has been described. Dehorne (1911) found the same to occur in the somatic mitoses of *Salamandra*. In addition, I have reason to believe that this phenomenon occurs in the chromosomes of the first spermatocyte of the *Tettigidæ*, while they are yet in the daughter-cell stage resulting from the last spermatogonial division. There it seems that the chromosomes, before entering upon the stages of side-to-side pairing in synapsis, are in reality definitely split along a longitudinal plane. Pairing takes place evidently between members that are already made up of two strands, and of course the four-part tetrad results.

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EXPLANATION OF PLATES I, II, AND III.

Drawings were made by aid of a camera lucida and are here reproduced at 2600 diameters. The pairs of autosomes are numbered, according to size, from 1 to 6. The normal "accessory" chromosome is numbered *5x*. The numeral *5* indicates that it ranks between the fourth and fifth autosome in size. *S* designates the deficient supernumerary "accessory" (?) chromosome.

FIGS. 1 to 5. Divisions in somatic cells each showing 14 choromosomes.

FIG. 1. Fat body cell. No. 5's are foreshortened.

FIG. 2. Cell from the follicular wall of the testis. Each large cromosome shows at the proximal end a peculiar somewhat knoblike portion, which seems to be less deeply stained. The chromosomes are woolly. The longitudinal split marked.

FIG. 3. Cell from follicle wall. Follicle itself was full of spermatozoa. Chromosome No. 5 is foreshortened. Each choromosome in figures 2 and 3 is split twice longitudinally. This may be seen by focusing on parts of chromosomes running vertically. See cross-section of No. 4 at arrow point.

FIG. 4. One of the daughter cells, in a late anaphase or telophase of a follicular cell. Distal ends of these chromosomes show the secondary split. That which takes place at the division which follows the resting stage into which this cell is about to pass.

FIG. 5. Cross-sectional view of a daughter group of chromosomes in the anaphase stage similar to figure 5. Chromosomes very long. The secondary split is quite visible. This cell will soon pass into the resting stage. Cell taken from wall in region of the neck of the follicle.

FIG. 6. Spermatogonium in metaphase stage of division. Fourteen chromosomes.

FIG. 7. Similar spermatogonium from another follicle.

FIGS. 8 to 21. Stages of first spermatocytes from early synapsis to division.

FIG. 8. "Sheaf stage," corresponding possibly to the "bouquet" stage of other authors. The numbers of each of the six pairs of autosomes are in parasynapsis at one end, probably distal, where six winding threads may be counted. The normal accessory chromosome (*5x*) and its deficient

mate (*s*) lie to one side of the nucleus coiled in corkscrew fashion, the spiral turning toward the right. Both point to what is probably the distal end of the cell.

FIGS. 9*a*, 9*b*. No. *5x* and *s* pairing in synapsis stages slightly later than figure 8.

FIGS. 10*a*, 10*b*. Similar stages. Each accessory shows a longitudinal split.

FIGS. 11*a*, 11*b*, 11*c*. Similar stages; all taken from one cyst. Accessory chromosomes usually lie near together, but may lie on opposite sides of the nucleus. Inequality of the two is apparent.

FIGS. 12, 13. Fine spireme stages following the bouquet stage. The accessory and its supernumerary mate may lie close together or on opposite sides of the nucleus. Both always lie on the nuclear membrane.

FIGS. 14*a* and 14*b*. Cell from two sections. Early prophase. Six pairs of autosomes form tetrads. The normal *5x* accessory and its mate near by. Note shortness of the supernumerary. Both are split.

FIGS. 15*a*, 15*b*, 15*c*. Three similar stages from other cells of the cyst. Both chromosomes split.

FIG. 16. Similar stage; complete cell. Chromosome No. 6 moved outward.

FIGS. 17 to 21. Division of first spermatocyte. Supernumerary going either with the accessory or to the opposite pole. In 65 to 75 per cent of the cases it lay near the accessory, but might not (figs. 20, 21). All other chromosomes of normal size and dividing normally. Chromosome No. 5 slightly displaced to right in figure 17.

FIGS. 22 to 25. Second spermatocyte division, polar view, showing six, seven and eight chromosomes, depending upon whether No. *5x* and *s* were absent or present, either one at a time or both.

FIGS. 26, 27. Second spermatocyte division, lateral views of the spindle, showing that all chromosomes divide. Six chromosomes in one, the *5x* and the *s* chromosome absent; eight in the other, both the *5x* and the *s* chromosome present.

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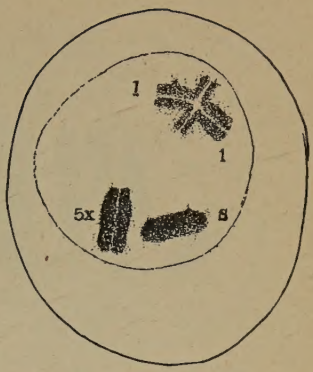

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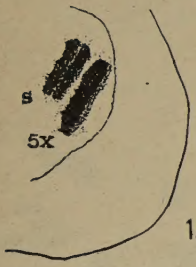

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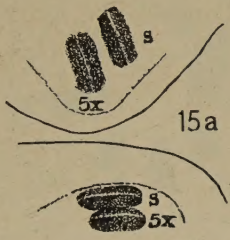

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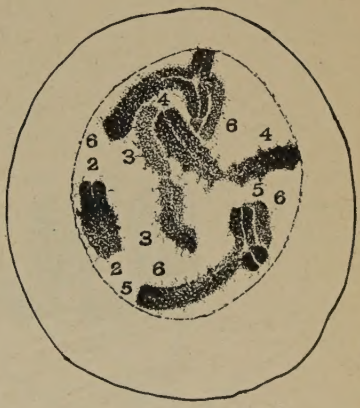

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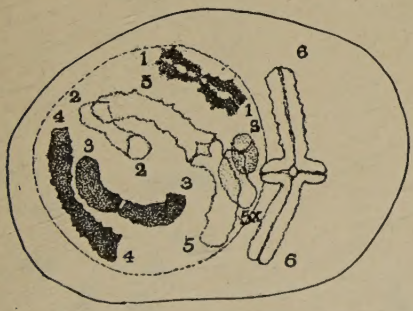

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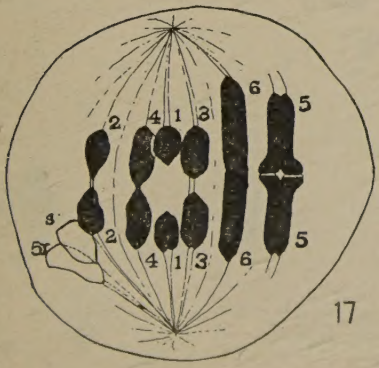

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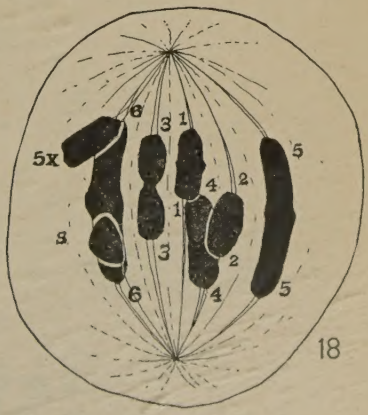

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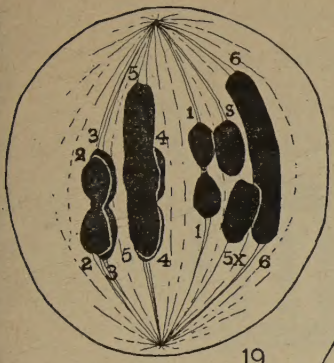

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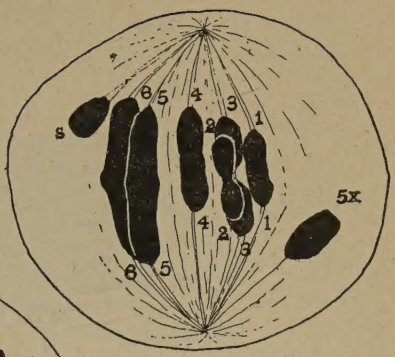

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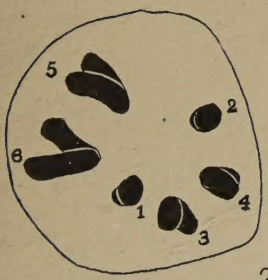

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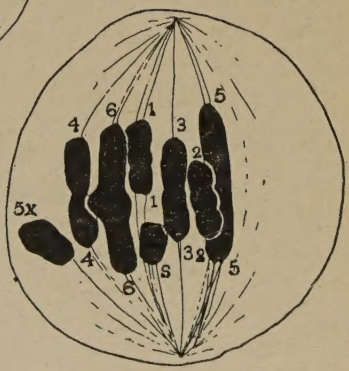

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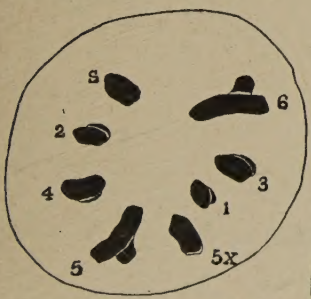

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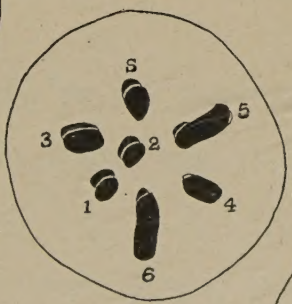

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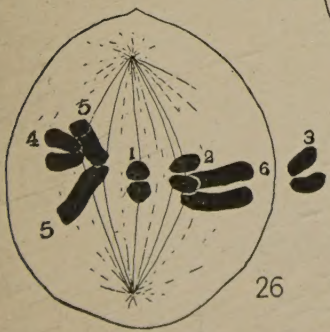

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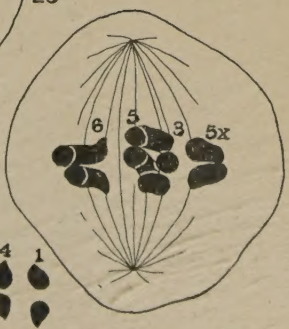
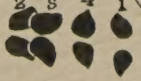
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